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Propogation of Juniperus for Conservation Plantings in the Great Plains

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PROPAGATION OF *JUNIPERUS* FOR CONSERVATION PLANTINGS IN THE GREAT PLAINS

by

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PROPAGATION OF *JUNIPERUS* FOR CONSERVATION PLANTINGS IN THE GREAT PLAINS

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Nursery managers have difficulties in producing consistent crops of eastern redcedar (Juniperus virginiana L.) and Rocky Mountain juniper (Juniperus scopulorum Sarg.), two of the most widely planted conservation species in the Great Plains. This study examined ways in which germination of these two species could be enhanced either through alteration of dormancy or alteration of the plantable seed lot. Three seed lots of eastern redcedar from the Great Plains and five seed lots of Rocky Mountain juniper from the eastern Rocky Mountains were used in the study. Treatments to alter dormancy included gibberellic acid (GA_3), potassium nitrate (KNO_3), osmotic priming (PEG), and tumbling seeds in a rock polisher. Seed separation techniques included separation by size and density (SD) and the Incubation, Dehydration, Separation (IDS) technique. Seed germination, as well as seed viability as determined by tetrazolium chloride (TZ), were measured for the various treatments. Leachate conductivity was examined as a possible inexpensive and simple substitute for TZ testing.

Significant increases in germination for the seed dormancy treatments occurred only in the GA_3 treatment, however, regardless of treatment germination overall remained extremely low (1.45%). The SD method was successful in separating out seed classes that significantly differed in quality as measured by seed viability. More interestingly, the

SD method significantly enhanced germination and germination + broken seed coats of all seed regardless of separation class (4.2 and 10.8% respectively) indicating it had an additional impact on dormancy. The lack of significance in separated groups attained using the IDS technique may have been due to the overall viability of the seed lots (65.3%), and, as such, should not be disregarded as a potential tool to enhance germination in these two species. The leachate conductivity method was found to be a reliable proxy for the more time consuming TZ method.

These results suggest a dual dormancy treatment (one that combines a treatment for both embryo and seed coat dormancy), is needed for these two species. Until this treatment is developed, nursery managers can still enhance the germination of eastern redcedar and Rocky Mountain juniper by using the leachate conductivity method to assess the initial quality of the seed lots and, then if necessary, use the SD technique to separate out a more viable population of seeds for planting.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. Background

1.1.1. Eastern redcedar

Eastern redcedar (Juniperus virginiana L.) is an important tree species in North America. Some of its many uses include food and shelter for wildlife, shelterbelt plantings for crop production and soil conservation, mine reclamation, and ornamental uses such as Christmas trees. The wood of the eastern redcedar is highly valued for its beauty, durability, and workability (Williamson 1957).

Eastern redcedar can be found in 37 states. The species' range includes all states east of the 100th meridian (Lawson 1990). Eastern redcedar also occurs in parts of Canada such as southern Ontario and the southern tip of Quebec (Williamson 1965). It can also be found in parts of New Brunswick and Nova Scotia in Canada (USDA FS 1955). The range of eastern redcedar is entirely encompassed between 29^0 to 45^0 north latitude and 69^0 to 102^0 west longitude (Van Haverbeke and Read 1976).

Extremes of temperature for eastern redcedar vary from -40^0C (-40^0F) in the central plains and Minnesota to $+46^0$C (115^0F) in the central and southern Great Plains. Elevation varies from sea level to over 1,524 m in western Nebraska and Kansas (Van Haverbeke and Read 1976). Precipitation in the range of eastern redcedar varies from 40.6 cm in the Great Plains to 152.4 cm in the southeast United States (Van Haverbeke

and Read 1976). The growing season varies from 120 days in North and South Dakota to 250 days in the southern Coastal Plain (Williamson 1965).

Eastern redcedar is most commonly found on poorly developed, shallow soils (Lawson 1990). Optimal site conditions for eastern redcedar are deep, moist, well drained alluvial sites, but it can be found on many sites with highly variable soil types and even dry rock outcrops. The species can be found on a wide range of soils from acidic soils to those soils derived from limestone. The pH of these soils can range from 4.7 to 7.8 (Arend 1950). On optimal sites, heights of 30 to 37 meters for mature trees have been measured (Van Haverbeke and Read 1976).

1.1.2. Rocky Mountain juniper

Rocky Mountain juniper (Juniperus scopulorum Sarg.) is another important tree species in North America. Along with eastern redcedar, Rocky Mountain juniper is also an important species for planting in shelterbelts. It is one of only 13 junipers native to North America. The wood is fine grained, with white sapwood and deep red heartwood with faint purplish and whitish streaks (Noble 1990). The wood of Rocky Mountain juniper has been used as fenceposts, furniture, cedar chests, and closet linings.

Rocky Mountain juniper has a large native range, however the distribution throughout its range is very scattered. Rocky Mountain juniper can be found throughout the western United States and Canada generally following the Rocky Mountains. It also appears in the Uinta and Wasatch Mountains in Utah and from the Grand Canyon in Arizona to the Black Mountains of southwestern New Mexico.

Temperature extremes for Rocky Mountain juniper vary from +43^{0}C (110^{0}F) to -37^{0}C (-35^{0}F) but minimum temperatures of approximately -22^{0}C (-8^{0}F) are more favorable for the species (Noble 1990). Elevation varies from near sea level to approximately 2,743 meters in the Rocky Mountains. Precipitation varies from 25 cm in its southern range to 84 cm in the northern Rocky Mountains. The growing season varies from 120 days in the northern Rocky Mountains to 175 days in Arizona and New Mexico (Noble 1990).

Rocky Mountain juniper is usually found on soils that are rocky, shallow, have a low moisture holding capacity, and are easily erodible. The pH of these soils is generally around 8.0 (USDA, SCS 1975). With these poor soil conditions, the trees commonly grow at a relatively slow rate. Optimal site conditions for Rocky Mountain juniper are in sheltered areas found in canyons and ravines (Noble 1990). The largest known Rocky Mountain juniper measures 198 cm in D.B.H. (Diameter Breast Height) and is 11 meters tall although some Rocky Mountain junipers can reach 15 meters in height.

1.2. Problem

Eastern redcedar and Rocky Mountain juniper are among the most widely planted conservation species in the Great Plains. In 1992, nearly 2.7 million Juniperus seedlings were distributed by Great Plains nurseries (Moench 1993). Due to problems associated with dormancy and seed germination, especially in Rocky Mountain juniper, nursery managers have difficulty producing a consistent crop of Juniperus seedlings. For example, the Oklahoma Department of Agriculture reports that typically only 16% of their redcedar seeds germinate during the first year after planting and their seed to saleable seedling ratio

is extremely low at 3% (Porterfield, personal communication). Germination of Rocky Mountain juniper is also unusually low at the Oklahoma nurseries. The low germination rates observed for Juniperus suggest that the current cultural practices are inadequate to overcome dormancy.

A more efficient method of germination must be found to supply the increasing demand for these conservation species. The current methods are expensive, labor intensive, and still fail to produce a consistent crop of seedlings. In the present study I considered two approaches to improving production of Juniperus from seed: 1) methods to break dormancy such as chemicals, hormonal applications, and physical treatments to enhance seed germination and 2) increasing the proportion of viable seed sown through seed separation techniques. Because Juniperus seed exhibit seed coat and embryo dormancy, I considered seed treatments related to both types of dormancy.

1.3. Objectives

The objectives of this project are to determine the effects of various treatments on germination and the ability of separation techniques to select out more viable populations of Juniperus seed from the Great Plains and Rocky Mountains. This report consists of four chapters. A review of literature is presented in Chapter 2. The results of the separation and germination experiments are presented in Chapter 3. Chapter 4 is a summary of results and conclusions along with recommendations.

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CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

In order to address the problems associated with the propagation of Juniperus, a knowledge of the biological mechanisms that influence the production of seedlings is needed. First, a discussion of general germination and dormancy is presented. This is followed by a more in depth look at the types of dormancy exhibited in eastern redcedar and Rocky Mountain juniper. Secondly, a review of current cultural practices for production of Juniperus seedlings is presented and discussed. Lastly, an array of literature is reviewed focusing on methods to overcome seed coat and embryo dormancy in seeds of eastern redcedar, Rocky Mountain juniper, and other tree and vegetable species.

2.2. Germination

Germination commences with the imbibition of water and the activation of metabolism in the embryonic tissue (Larcher 1995). The imbibition of the seed consists of three phases: an initial uptake of water, a lag phase, and a second uptake of water (Bewley and Black 1994). The first water uptake by the seed is due to the much lower water potential of the seed as compared to the soil. The lag phase of water uptake is characterized by metabolic events which are taking place in preparation for radicle emergence. Phase three of water uptake occurs after the radicle has protruded and germination is complete. Dormant and non-dormant seeds experience phase one and two

water uptake but only non-dormant germinating seeds can achieve phase three of water uptake.

The lag phase of water uptake is a crucial component of the germination process. The initial uptake of water triggers hormones to stimulate enzyme formation and activity. Gibberellin, a plant hormone, stimulates the synthesis of enzymes such as amylase, lipase, isocitrate lyase, and RNA polymerase in seeds (Kramer and Kozlowski 1979). Once hydrolyzed, these enzymes act on the storage reserves in the seed which provide the energy for cell elongation (Kozlowski et al. 1991). The cell elongation occurs in the radicle which pushes it into the soil. This is followed by the cotyledons emerging from the soil. Germination of eastern redcedar and Rocky Mountain juniper follows this epigeous pattern (USDA FS, 1948).

Eastern redcedar and Rocky Mountain juniper have relatively low storage reserves as compared to other tree species. This is evident by the small size of the seeds. The average number of seeds per kilogram for eastern redcedar is 19,800 while the average for Rocky Mountain juniper is around 12,300 (Johnsen and Alexander 1974). After the storage reserves are depleted, the cotyledons will provide the carbohydrates needed for growth once they emerge from the soil. Cotyledons are relatively short lived and are relieved by the first true foliage leaves. The first growth season is used mainly to develop the root system (Ferguson et al. 1968). While some foliage growth begins in the first growing season, most occurs during the second and subsequent growing seasons.

2.3. Dormancy

The very poor and erratic germination of eastern redcedar and Rocky Mountain juniper is primarily due to the seeds being dormant (Van Haverbeke and Comer 1985; Rietveld 1989). Dormancy is the inability of a seed to germinate, even under conditions that are normally considered favorable for germination (Bewley and Black 1994). Dormancy can be caused by the seed coat, the embryo, or a combination of the two (Bewley and Black 1982). Coat imposed dormancy can be attributed to several factors: 1) the impermeability of the seed coat to water and gases; 2) the seed coat preventing germination inhibitors from leaving the embryo; 3) the seed coat supplying inhibitors to the embryo; or 4) the mechanical prevention of radicle extension (Kelly et al., 1992). Scarification is often needed to break seed coat dormancy. In the case of embryo dormancy, a physiological trigger such as a hormone, temperature, or light is required to break the dormancy. Seeds of Juniperus have both types of dormancy (Pack 1921). Djavanshir and Fechner (1976) state that the low germination of Juniperus seeds is due to chemical factors in the embryo and physical factors in the seed coat. A confounding problem with Rocky Mountain juniper is that the seeds may be immature (Rietveld 1989). Rocky Mountain juniper seeds take two years to mature on the tree. When seeds are collected some immature seeds can be inadvertently collected with the mature seeds.

The Great Plains can be a very hostile environment for a plant to germinate and survive. By Kigel's (1995) definition, the Great Plains could be considered a vast desert where evaporation exceeds precipitation and large fluctuations occur between maximum and minimum temperatures throughout the year. Precipitation in the Great Plains is

relatively low and occurs irregularly. Dormancy is an adaptation by species to ensure survival in this risky environment. Dormancy helps spread the risk so that one failure to germinate will not be detrimental to the species (Cohen 1966).

A downside of dormancy is that viability of buried seeds may decline exponentially over time. Bewley and Black (1994) reported that seeds of some species can remain viable for hundreds of years, however this is not the case for eastern redcedar and Rocky Mountain juniper. Holthuijzen and Sharik (1984) found that only 5.5% of buried eastern redcedar seeds were viable after 14 months. The initial viability of the seeds was approximately 90%. This is a large loss in viable seeds and implies that if the seeds do not germinate in the first or second year after dispersal, there is an extremely low chance that the seeds will ever germinate. Eastern redcedar's survival strategy is to produce large numbers of seeds that are randomly deposited by birds. Some seeds will then be deposited in areas where they can establish when conditions are favorable (Schmidt 1991).

It is important to understand the mechanisms of dormancy before attempting to overcome the problem of dormancy. Amen (1968) suggests that seed dormancy can be separated into 4 phases: 1) inductive, 2) maintenance, 3) trigger, and 4) germination. Induction is characterized by a decrease in hormone levels, maintenance is a period of partial metabolic rest, trigger is when the seeds are sensitive to specific environmental cues, and germination is marked by increased hormone and enzyme activity culminating in growth.

Two types of dormancy that occur in seeds are primary and secondary dormancy (Bewley and Black, 1985). Primary dormancy is when the seeds are dispersed from the

mother plant in a dormant state. These seeds can be dispersed with different degrees of dormancy, a phenomenon known as polymorphism. Secondary dormancy occurs when a hydrated mature seed experiences adverse environmental conditions such as extremes in temperature, moisture, light, or a combination of these factors. Although these seeds are ready to germinate, they will lapse back into a dormant state until conditions are more favorable for germination.

In addition to primary and secondary dormancy, differences in maternal parents can also account for the degree of dormancy a seed possesses. Jones (1989) found that germination percentages differed significantly between seeds of East African pencil cedar (Juniperus excelsa M. Bieb.) from different maternal parents. The final germination percent ranged from 18 to 60%. Also, Rees (1994) found that increases in adult longevity select against seed dormancy. As the parent ages, the crop of seeds dispersed yearly will be less dormant than when the parent was younger. Although not dealt with in this study, maternal age could play an important role in germination of eastern redcedar and Rocky Mountain juniper.

2.4. Current nursery practices

Many studies have been conducted on eastern redcedar and a few studies have been conducted on Rocky Mountain juniper to determine the most efficient method to germinate the seeds in field and laboratory conditions. Currently, the most successful laboratory method to germinate eastern redcedar seeds is a 96 hour soak in citric acid (10,000 ppm), with 6 weeks of moist-warm stratification (24^{0}C) and 10 weeks of moist-cold stratification (5^{0}C) (Van Haverbeke and Comer 1985). Conditions needed to

germinate Rocky Mountain juniper seeds are warm-moist stratification (20°C night and 30°C day) for 45-90 days followed by cool-moist stratification (5°C) for 30-120 days (Johnsen and Alexander 1974).

The most common method for germinating seeds in the field is by covering the seedbed with clear plastic and shade frame after sowing stratified seeds in the fall. After overwintering, the seeds begin to germinate under plastic as temperatures become favorable in the spring. The nursery manager must watch the seed closely to determine when to lift the shade frame and plastic. The problem with this method is that it is expensive, labor intensive, and still fails to produce a consistent crop of seedlings. In addition, late frosts after lifting the plastic mulch may cause considerable damage to the newly germinated seedlings (Fleege 1994, personal communication). It is for these reasons that our understanding of the factors controlling dormancy in these species must be improved and more efficient methods to increase germination must be developed.

A relatively new nursery germination technique for eastern redcedar and Rocky Mountain juniper is to stratify the seeds for 16 weeks but sow the seeds in the spring instead of the fall. Spring sowing improves the germination rate to approximately 60% while lowering the expense and labor that accompanies fall sowing (Oklahoma Forest Regeneration Center 1994, personal communication). While spring sowing of eastern redcedar and Rocky Mountain juniper has not been extensively tested, it does have the potential of becoming a valuable germination technique for nursery managers.

2.5. Embryo dormancy

Embryo dormancy is caused by an accumulation of germination inhibitors inside the seed. This accumulation of inhibitors can occur in the cotyledons or in the entire embryo. Bewley and Black (1994) state that there is evidence that dormancy in apple seeds is caused by abscisic acid (ABA) which is present in the cotyledons. If the cotyledons are removed (ABA removed), germination occurs. If the cotyledons are left intact, the result is minimal or no germination. Embryo dormancy caused by germination inhibitors in the entire embryo can be released by leaching which flushes the inhibitors out of the seed. Three methods that have shown success in breaking embryo dormancy are stratification, hormonal applications, and priming (Khan 1977). Currently, the most accepted method for breaking embryo dormancy in eastern redcedar and Rocky Mountain juniper is a warm moist stratification followed by a cool moist stratification (Van Haverbeke and Comer 1985; Johnsen and Alexander 1974).

2.5.1. Stratification

Stratification (chilling of the seeds) mimics natural overwintering to promote seed germination. In Juniperus, this is achieved through a warm moist stratification period followed by a cool moist stratification period. In other species, the duration of stratification may be different. In some species, the warm moist stratification period may be omitted and only a cool moist stratification period is necessary. For example, herbaceous species may need only a short stratification consisting of a few days while woody species may need to be stratified for months.

Stratification has promoted germination for many species that exhibit partial or full embryo dormancy. Stratification of western juniper (Juniperus occidentalis Hook.) and Utah juniper (Juniperus osteosperma [Torr.] Little) in aqueous solutions with near saturation of the solution with oxygen increased germination to approximately 50% (Young et al., 1988). In the same study, treatments using aqueous solutions of 0.289 mol L^{-1} gibberellic acid (GA_3) improved germination of western juniper to better than 80%. Similar results were obtained (69% germination) with seeds of Alaska-cedar (Chamaecyparis nootkatensis D. Don) with a 60 day warm stratification followed by a 90 day cold stratification (Pawuk 1993).

Pine seeds have also shown marked increases in germination following stratification. Korean pine seeds (Pinus koraiensis Sieb. et Zucc.), which possess embryo and seed coat dormancy similar to eastern redcedar and Rocky Mountain juniper, will germinate after three months of stratification at a constant temperature of 3^0C (Qi et al., 1993). Flannigan and Woodward (1993) found that temperatures of 15^0C or greater were required to germinate 80% or more of red pine seeds (Pinus resinosa Ait.). Barnett (1993) found that the more adverse the germination conditions were (15^0C was adverse while 22°C was ideal), the more important it was to have longer stratification periods in shortleaf pine seeds (Pinus echinata Mill.). The longer the stratification period, the closer the seeds were to germinating. This enables the seeds to germinate in spite of the adverse germination conditions.

The mechanism whereby stratification reduces dormancy is poorly understood. It was previously believed that ABA levels decline during stratification and that this ABA

decline was responsible for the increase in germination. Subbaiah and Powell (1992) measured ABA levels in apple seeds (Malus domestica Borkh.) to determine the effect chilling had on levels of ABA in the seeds. It was confirmed that chilling was not related to ABA changes during stratification in warm (20^{0}C) and cold (5^{0}C) stratification experiments. ABA content dropped rapidly and nearly identically under both temperature stratifications, but germination occurred only after cold stratification. The decline in ABA is mostly due to leaching from the seed coat and nucellar membrane while the ABA content of the embryo remained fairly constant.

2.5.2. Hormones

Several authors have suggested that hormones (or hormonal balance) play a key role in controlling germination. By applying hormones to a seed, mainly gibberellic acid (GA), the balance of hormones inside of the seed may be altered. If a germination promoter is applied (GA), it can counterbalance the effect of an overabundance of a germination inhibitor (ABA) inside of the seed. With more germination promoter than germination inhibitor inside of the seed, embryo dormancy may be broken and germination may commence.

Gibberellic acid (GA_3) is one promoter that has proven to be successful in breaking embryo dormancy. Geneve (1991) found that GA_3 (50 μM) increased germination over the control (2±1.7) to 28±4.8 in eastern redbud (Cercis canadensis var. canadensis L.) seeds. Singh (1989) found that treatments with GA_3 also increased germination over controls. Soaking seeds in solutions of 2160μM for 24 hours, 1440μM for 24 hours, and 480μM for

72 hours increased germination over controls of 19.75, 19.25, and 19.75% respectively for spruce seeds (Picea smithiana [Wall.] Boiss.)

Infusion of growth regulators in seeds of ornamental plants from 16 species representing 10 plant families with a taxonomic distribution from Asteraceae to Ramunculaceae significantly improved germination in 10 of the experimental species (Persson 1993). The infusion of growth regulators were with 1μM or 10μM gibberellic acid or with a concentration of 1μM gibberellic acid, 0.5μM kinetin and 1μM Ethrel (2-chloroethanephosphonic acid) dissolved in acetate. In all cases, germination rates increased over the control. The most effective treatment for increasing germination percentage was gibberellic acid at a concentration of 10μM.

In a study done by Lewandowska and Szezotha (1992), gibberellin, kinetin (Kin), and spermine (Spm) were applied to seeds of common ash (Fraxinus excelsior L.) to determine the effect of hormones on breaking dormancy. The treatments were applied as follows: GA_3, Spm, Kin, Kin + Spm, GA_3 + Spm, and water as a control. If the treatments were applied before warm stratification, they had an inhibitory effect on dormancy breakage and germination. Seeds in the water treatment germinated at 80% while the highest germination rate for other treatments was 50% for the seeds treated with GA_3. If the treatments were applied before cold stratification, they accelerated dormancy breakage but lowered germinability as compared to the control. Seeds treated with water germinated to approximately 80% while the highest other treatment, Spm, germinated to around 70%. Kinetin was also found to increase germination of East African pencil cedar seeds from 47% for controls to 63% for treated seed (Jones 1989). Pinfield and

Gwarazimba (1992) found similar results for kinetin treatments in seeds of Norway maple (Acer platanoides L.).

2.5.3. Potassium nitrate

It is believed that potassium nitrate (KNO_3) in combination with light promotes gibberellin synthesis (Mayer and Poljakoff-Mayber 1989). Gibberellin synthesis is known to break dormancy in seeds (Nadakavukaren and McCracken 1985). While studying the effects of KNO_3 on germination of wild oat (Avena fatua L.) seed, Hilton (1985) made an interesting discovery. She found that KNO_3 significantly increased germination from 18% for the control to 64% with 200 mol m^{-3} KNO_3. She also concluded that germination stimulation was dependent upon the quality of the light environment. The higher the Pfr/P ratio, the higher the germination rate.

Several authors have utilized KNO_3 to enhance germination rates in grass and weed species (Dickens and Moore 1974; Maguire and Steen 1971; and Young et al. 1992). Nagau and Furutani (1986) found that germination of Carica papaya (L.) was enhanced by density separation with sucrose solutions followed by a KNO_3 application. The germination percentage for the control was 35.9% while the germination percentage for the KNO_3 treatment was 87.8%. Concentrations of KNO_3 for germination testing often range from 0.1 to 0.2% in water solutions as recommended by the Association of Official Seed Analysts (1994).

2.5.4. Priming

Priming is another method that has been used to break embryo dormancy. Priming has been used to increase total germination and rapidity of germination in seeds of species

that have unreliable germination rates. As the name implies, priming prepares the seeds for germination. Priming allows all the processes of germination to proceed except radical emergence. This is achieved by lowering the total water potential of the solution during the initial hydration of the seeds. Two methods of priming are osmotic and matric priming. Osmotic priming utilizes an osmoticum such as polyethylene glycol or sodium chloride to lower the total water potential while matric priming utilizes a solid barrier to lower the total water potential. Both methods reduce the amount of water that is readily available for uptake by the seeds.

Osmotic priming using polyethylene glycol (PEG) has been demonstrated to improve germination in a number of species. Hallgren (1989) found that osmotic priming with aerated solutions of polyethylene glycol increased final amount and rapidity of germination for loblolly (Pinus taeda L.) and shortleaf pines, but was generally detrimental to germination of slash pine (Pinus elliottii Engelm.) seeds with one exception. At 15^{0}C germination temperature, Hallgren found that unprimed slash pine seed that was not stratified would not germinate but would germinate to 29% after priming.

Owen and Pill (1994) reported that the final percentage germination of asparagus (Asparagus officinalis L.) seeds increased from 48% for unprimed seeds to 74% for primed seeds. This was at a high osmotic stress of -0.4 MPa. Pill et al. (1994) also showed that germination could be enhanced by osmotic and matric priming in seeds of the purple coneflower (Echinacea purpurea L. Moench). With a cool emergence temperature, the time for 50% emergence was 7.3 days for PEG (osmotic), 7.2 days for vermiculite (matric), and 10.0 days for non-primed seeds. Also, the time between 10% and 90%

germination for the treatments was 5.4, 5.5, and 7.7 days respectively. The final germination rate for the 3 treatments were 81, 78, and 58%.

The effect of the depth of seed immersion in PEG solutions ranging from 0 to -1.5 MPa was studied by Hardegree and Emmerich (1994) with respect to germination of grass seed. The depth of immersion ranged from 0, 1, 3, or 5mm. Total germination (94%) and germination rate (0.6 days to 50% germination) were best under a 1mm solution of PEG. Hardegree and Emmerich (1994) attribute the lower germination rates of the deeper immersion depths to decreased oxygen availability.

Although priming increases germination in some species, the mechanism whereby germination rates are increased is poorly understood. Priming appears to act as a physiological trigger to initiate biochemical activity in the seed. Smith and Cobb (1991) found that soluble protein levels increased 115% in primed pepper seeds (Capsicum annuum L.), and the uptake and incorporation of labeled amino acids into the acid insoluble fraction increased throughout the priming treatments. Also, aldolase and isocitrate lyase activities increased with imbibition and were 61% and 56% higher respectively in primed seeds when compared to the water imbibed controls after 12 days.

In a separate study by Smith and Cobb (1992), pepper seeds were primed in NaCl solutions for twelve days and then either re-dried or re-dried and germinated in double distilled water to determine what effect these treatments had on protein and respiration levels during these processes. Both seed treatments retained higher soluble protein levels, aldolase activity, and isocitrate lyase activities when compared to the controls. In primed re-dried seeds, glucose-6-phosphate dehydrogenase (G6P) activity was 50% higher than in

control seeds, while 6-phosophogluconate dehydrogenase (6PG) remained the same and alcohol dehydrogenase (ADH) activity decreased by 44%. G6P and 6PG activities of primed, re-dried, germinated seeds remained the same as the control seeds. ADH levels became so low on these seeds that it could not be detected.

2.6. Seed coat dormancy

Several experiments have been conducted on seeds of eastern redcedar and Rocky Mountain juniper to break seed coat dormancy with limited success. Van Haverbeke and Comer (1985) soaked seeds in hydrogen peroxide, sulfuric acid, and citric acid in an attempt to break seed coat dormancy with limited success. Several methods have been found that can break seed coat imposed dormancy in other species. Some of these are microbial action in the soil, gut passage, extreme temperatures, cracking of the testa during fires, and exposure to solar radiation (Boesewinkel and Bouman 1995). All of these methods exert a scarification force on the seeds which helps to soften or crack the seed coat and allow germination to proceed. Scarification is a term used to describe the abrasion of the seed coat. This abrasion in turn will allow the embryo to germinate by initiating water and gas exchange or reducing the amount of force the embryo must exert to break the seed coat.

2.6.1. Mechanical scarification

Mechanical scarification such as hot wire methods and air gun scarifiers are often used in poorly developed countries or where chemicals are expensive or unavailable. Masamba (1994) found that germination of three species of Acacia seeds known to have seed coat dormancy increased to at least 80% by treating the seeds using a hot wire

method. This method basically burns the seed coat to scarify the seeds. The highest germination from the hot wire method came from a duration of 5-10 seconds.

Lehmann lovegrass seed (Eragrostis lehmanniana Ness.) germination was also increased by mechanical scarification and after-ripening of the seeds (Hardegree and Emmerich 1993). Thirty-four weeks after harvest, germination of scarified seed ranged from 42 to 83% while the best germination of nonscarified seed was only 9%. In a study by Jones and Nielson (1992) on Indian ricegrass seed (Oryzopsis hymenoides [Roem. and Schult.] Ricker), seeds were scarified with an air-gun, then stored for one year at 5^0C, prechilled at 5^0C for 3 weeks, or used as a control. Air-gun scarification is a method in which seeds are air forced through a cylinder lined with emery cloth (Booth and Griffith 1984). The air-gun scarifier improved germination in 12 of 13 undamaged seedlots from 9.5 to 29.7%. Prechilling the seeds improved germination in 10 of the 13 seedlots from 8.0 to 22.8%.

Another method of mechanical scarification is to cut or nick the seed coat. In a study of epicotyl and hypocotyl germination of eastern redcedar and Rocky Mountain juniper, Djavanshir and Fechner (1976) found that cutting the seed bases (hilum) resulted in epicotyl development within 3 to 9 days. Todd-Bockarie et al. (1993) and Todd-Bockarie and Duryea (1993) have also shown significantly increased germination rates for West African Laburnum (Cassia suberiana DC.) and velvet tamarind (Dialium guineense Willd.) respectively by nicking the seed coats.

2.6.2. Mechanical and chemical scarification combined

Eastern redcedar seeds can pass unharmed through the digestive tract of avian dispersers in around 30 minutes in most cases (Holthuijzen and Sharik, 1985b). The seed receives a chemical scarification (acid) along with a mechanical scarification (the churning of the gizzard). Bird-passed seeds showed 1.5 to 3.5 times greater germination than manually depulped seeds. Holthuijzen and Sharik (1985a) observed that 27.6 to 55.0% of seeds that passed through birds germinated while germination for the control was 16.1%. Seeds collected from avian dispersers showed an average of 40% total germination. Dispersers included the yellow-rumped warbler, cedar waxwing, American robin, and the eastern bluebird.

2.7. Separation

The prior discussion has dealt with methods that break seed dormancy. We will now examine separation methods which have shown promise in increasing germination rates by separating out a more viable subsample of seeds to be planted. In addition, these methods may also be used to determine the initial viability of a given seed lot.

2.7.1. Size and density

Seed separation by size and density is an effective method to separate viable from non-viable seeds (Gonzalez 1993; Khademi et al. 1993). In general, as seeds mature, size and density increase. Therefore, theoretically the larger and denser seeds will produce a more viable seed lot and the immature seeds may be culled before planting. These separation procedures are relatively easy to perform and provide a fast and efficient method to increase the viability of the seeds to be sown.

Khademi et al. (1993) showed that as the density of Primula acaulis (L.) rose, the percent viability and soluble protein contents increased. The densities ranged from 1.10 to 1.18 g/cm^3. The percent viability and soluble protein contents rose from 8 to 90% and from 26 mg/g to 38 mg/g as the seed density increased. Wang et al. (1994) showed that as seed mass increased in black spruce (Picea mariana Mill.) seeds, the seedling survival rate also increased. At an initial seed mass of 0.55 mg, survival rate was approximately 42% while an initial seed mass of 1.5 mg produced approximately 75% survival rate for seedlings. In contrast, Douglas et al. (1994) reported that an emergence rate from 7 to 11 days after seeding and germination was significantly greater for small-light seed than for small-dense or normal mix seed of soft white winter wheat (Triticum aestivum L.).

Mutha et al. (1994) separated seeds of Prosopis juliflora (SW.) into 7 weight classes ranging from 1.07 to 4.00g per one hundred seeds. They concluded that the large, heavier seeds showed earlier germination and a higher percent germination than the lighter seeds. The percent germination ranged from 26.2% for the lightest seeds and 87.4% for the heavier seeds. In contrast, Reich et al. (1994) found that in Scots pine, where the average seed mass per 1000 seeds ranged from 4.4 to 9.0g in European seed sources, germination showed a linear decrease in germination rates with increased seed mass.

2.7.2. IDS

The IDS technique is based on the principle that viable seeds, once hydrated, release moisture more slowly than unfilled or non-viable seeds. The procedure involves hydrating the seeds (Incubation), drying the seeds for a specified time (Dehydration), and then placing the seeds in water and discarding the seeds that float (Separation). Viable

seeds contain functional membranes which regulate water loss while non-viable seeds may contain damaged or unstable membranes (Bewley and Black 1994). The damaged or unstable membranes allow for more rapid water loss as compared to functional membranes. Following the dehydration period of the IDS technique, viable seeds (slow moisture release) sink in tap water while non-viable seeds (rapid moisture release) float. The IDS technique has proven to be effective in separating viable from non-viable seeds for a number of woody species.

Vozzo and Singh (1994) found that germination of Pinus roxburghii (Sarg.) could be enhanced using the IDS technique. By immersing the seeds in sucrose solutions with a specific gravity of 1.04 for 4 hours, they found that 72% of the seeds sank while 28% of the seeds floated. Of the 72% of seeds that sank, 94% of these seeds germinated. Of the 28% of the seeds that floated in the solution, only 50% of the seeds germinated. The control for the experiment germinated at 73%.

Simak (1984) found that for a sample of lodgepole pine (Pinus contorta Dougl.) seeds that typically germinated to a 67% capacity, almost all of the 33% that did not germinate were found to be dead using the IDS technique. Increases in germination attributed to the IDS technique have also been reported for Scots pine (Pinus sylvestris L.) (increased from 33 to 95%) (Bergsten 1988), lodgepole pine (increased from 37 to 75%) (Downie and Wang 1992), and white spruce (increased from 50.2 to 86%) (Downie and Bergsten 1991).

2.7.3. Leachate conductivity

Leachate analysis has shown a relationship between seed health and electrical conductance (Bonner 1988). In general, as seed quality decreases, the amount of solutes leached from the seeds increases. The increased leaching is likely due to the immaturity of cell membranes or the rupturing of the cell membranes during the initial imbibition period. Imbibition of all seeds is accompanied by a loss of solutes into the surrounding solution because membrane integrity is incomplete. These solutes consist of sucrose, organic acids, ions, amino acids, and proteins (Bewley and Black 1994). In viable seed, membranes either revert to their most stable configuration or are repaired over time. In non-viable seed, such repair mechanisms may be absent, inefficient, or the membranes could be beyond repair. As a result, non-viable seeds continue to leak electrolytes due to damaged membranes. For example, Vozzo (1994) found that a seed lot of loblolly pine that germinated at 80% averaged 10.5 μmhos/gram seed weight while a seed lot of loblolly pine that germinated at only 1% averaged 60.2 μmhos/gram seed weight.

Sahlen and Gjelsvik (1993) concluded that leachate conductivity measurements can be very useful for determination of Scots pine seed maturity. Leachate conductivity as well as the leakage of inorganic phosphorus and carbohydrates decreased during ripening with increasing anatomical and physiological maturity until the approximate time for cessation of anatomical development. Bonner and Vozzo (1986) showed that leachate conductivity data could be used to predict laboratory germination of loblolly pine to within 6.5% and slash pine to within 7.2%.

2.8. Additional factors affecting germination

Boyle and Kuser (1994) found that Atlantic white-cedar (Chamaecyparis thyoides L.) germination was increased by using a 16-hour daylength rather than a 10-hour daylength. Germination increased from 0.7% to 31.9% by utilizing the 16-hour photoperiod during germination. The study was performed on fresh seed. A possible explanation the authors give for this increase in germination is that the cedar tree is an intolerant species and it needs high light levels to trigger the germination process.

Vozzo et al. (1994) found that the surface texture of dormant and germinated Korean pine seeds was different. The dormant seeds had more wax and lipids on the surface than the germinated seeds. The wax and lipid content on the surface of the germinated seeds decreased during stratification allowing increased imbibition and germination over dormant seeds.

2.9. Conclusion

From the literature review, three areas were identified as promising avenues to enhance production of eastern redcedar and Rocky Mountain juniper. These three areas consist of methods to break seed dormancy, seed separation techniques to create a more viable subsample, and a method to quickly assess the initial quality of a seed lot. These methods not only have shown promising results in other species, but they are also readily adaptable for use by nursery managers.

Seed dormancy was tackled using four different treatments; three treatments to break embryo dormancy (GA_3, KNO_3, and PEG) and one treatment to break seed coat dormancy (tumbling seeds in a rock polisher). In order to separate out more viable

subsamples, two separation methods (seed separation by size and density and the IDS technique) were examined. Finally, the leachate conductivity technique was compared to the tetrazolium chloride (TZ) method to determine if it could serve as a quick and reliable technique for assessing the initial viability of a seed lot.

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CHAPTER 3

INCREASING GERMINATION RATES OF EASTERN REDCEDAR AND ROCKY MOUNTAIN JUNIPER FOR CONSERVATION FORESTRY IN THE GREAT PLAINS

To be submitted to Tree Planter's Notes.

Abstract

Nursery managers have difficulties in producing consistent crops of eastern redcedar (Juniperus virginiana L.) and Rocky Mountain juniper (Juniperus scopulorum Sarg.), two of the most widely planted conservation species in the Great Plains. This study examined ways in which germination of these two species could be enhanced either through alteration of dormancy or alteration of the plantable seed lot. Three seed lots of eastern redcedar from the Great Plains and five seed lots of Rocky Mountain juniper from the eastern Rocky Mountains were used in the study. Treatments to alter dormancy included gibberellic acid (GA_3), potassium nitrate (KNO_3), osmotic priming (PEG), and tumbling seeds in a rock polisher. Seed separation techniques included separation by size and density (SD) and the Incubation, Dehydration, Separation (IDS) technique. Seed germination, as well as seed viability as determined by tetrazolium chloride (TZ), were measured for the various treatments. Leachate conductivity was examined as a possible inexpensive and simple substitute for TZ testing.

Significant increases in germination for the seed dormancy treatments occurred only in the GA_3 treatment, however, regardless of treatment germination overall remained extremely low (1.45%). The SD method was successful in separating out seed classes that significantly differed in quality as measured by seed viability. More interestingly, the SD method significantly enhanced germination and germination + broken seed coats of all seed regardless of separation class (4.2 and 10.8% respectively) indicating it had an additional impact on dormancy. The lack of significance in separated groups attained using the IDS technique may have been due to the overall viability of the seed lots

(65.3%), and, as such, should not be disregarded as a potential tool to enhance germination in these two species. The leachate conductivity method was found to be a reliable proxy for the more time consuming TZ method.

These results suggest a dual dormancy treatment (one that combines a treatment for both embryo and seed coat dormancy), is needed for these two species. Until this treatment is developed, nursery managers can still enhance the germination of eastern redcedar and Rocky Mountain juniper by using the leachate conductivity method to assess the initial quality of the seed lots and, then if necessary, use the SD technique to separate out a more viable population of seeds for planting.

3.1. Introduction

Eastern redcedar and Rocky Mountain juniper are among the most widely planted conservation species in the Great Plains. Seedlings of both species are planted in shelterbelts, living fences, and other conservation forest systems. In 1990, nearly 2.7 million Juniperus seedlings were distributed by Great Plains nurseries (Moench 1993). Due to problems associated with dormancy and seed germination, especially in Rocky Mountain juniper, nursery managers have difficulty producing a consistent crop of Juniperus seedlings to meet the demand. For example, the Oklahoma Department of Agriculture reports that typically only 16% of their eastern redcedar seeds germinate during the first year after planting (Porterfield 1993, personal communication). Germination of Rocky Mountain juniper is also unusually low at the Oklahoma nurseries. In a study we conducted at Bessey Nursery (USDA-FS, Halsey, NE) where we examined a number of cultural treatments to enhance germination in eastern redcedar and Rocky

Mountain juniper, germination rates never exceeded 20% (Cregg et al. 1994). The maximum germination rate we obtained for Rocky Mountain juniper in another study looking at a spring sowing period for these species was 17% (unpublished data). Obviously the low and erratic germination rates create a problem for nursery managers trying to meet specific production targets.

Due to the increasing demand for these two species, new methods of enhancing germination must be found that will enable the growers to efficiently supply this demand. Three approaches were identified as promising avenues to enhance production of eastern redcedar and Rocky Mountain juniper: trying to reduce seed dormancy, separating out a more viable subsample of seeds for planting, and assessing the initial viability of the seed lot to determine if additional measures are needed.

3.1.1. Seed dormancy

A major problem in being able to consistently produce a reliable crop of eastern redcedar and Rocky Mountain juniper is the ability to overcome the high degree of seed dormancy normally exhibited in these species (Van Haverbeke and Comer 1985; Rietveld 1989). Dormancy is the inability of a seed to germinate, even under conditions that are normally considered favorable for germination (Bewley and Black 1994). Seeds of eastern redcedar and Rocky Mountain juniper possess both seed coat and embryo dormancy (Pack 1921).

Of the current methods to treat embryo dormancy, treatment with gibberellic acid (GA_3), potassium nitrate (KNO_3), and osmotic priming with polyethylene glycol (PEG) are three promising methods not yet tried on eastern redcedar and Rocky Mountain juniper.

Gibberellic acid has proven to be effective for breaking embryo dormancy in several vegetable and tree species (Singh 1989, Durrant and Mash 1991). When applied to the seeds, GA_3 alters the hormonal balance of the embryo, thereby promoting germination. Application of KNO_3, in combination with light, is believed to promote gibberellin synthesis (Mayer and Poljakoff-Mayber 1989). Gibberellin synthesis has been demonstrated to break dormancy in seeds (Nadakavukaren and McCracken 1985). Osmotic priming with aerated solutions of PEG works by regulating water uptake by the seeds which promotes both overall germination and uniformity of germination (Halgren 1989, Owen and Pill 1994).

Seed coat dormancy is usually overcome by scarifying or wearing down the seed coat. Methods available range from using acid to chemically scarify the seed to physically damaging the seed coat (Bewley and Black 1994). It has been shown that the grinding action of a bird's gizzard promotes germination of eastern redcedar and Rocky Mountain juniper (Holthuijzen and Sharik 1985). A rock polisher would be an easy and inexpensive way to physically scarify the seed of these two species.

3.1.2. Seed separation and viability

A confounding problem in germinating seeds of Rocky Mountain juniper is that the seeds may be immature (Rietveld 1989). Rocky Mountain juniper seeds take two years to mature on the tree. When seeds are collected some immature seeds can be inadvertently collected along with the mature seeds resulting in a lowered overall viability of the seed lot. Using a method such as separation by size and density (Ganzalez 1993; Khademi et al. 1993) or the Incubation, Dehydration, Separation (IDS) technique (Downie and Wang

1992) should greatly enhance germination success. In theory, the lower the density of the seeds, the more likely the seeds are immature or empty. With the IDS technique, the slower moisture is released from the seeds, the greater possibility the embryo is intact and functional (more viable).

Currently, viability is routinely measured by staining of the embryo with tetrazolium chloride (TZ) and doing a visual assessment. Leachate analysis has shown a relationship between seed health and electrical conductance (Bonner 1988). Viable seed are assumed to leach less solutes than non-viable seed. It may therefore be possible to use the leachate conductivity as a quick and inexpensive indicator of seed viability instead of TZ testing.

3.2. Materials and methods

3.2.1. Plant materials

To obtain a representative cross-section of seed used in current nursery production, we requested seed from operational seed lots of the Western Forest and Conservation Association members. Seeds were collected on different dates and from different locations throughout the Great Plains (Table 1). Eight separate seed lots were used in the study, three seed lots of eastern redcedar and five seed lots of Rocky Mountain juniper. These seed lots were chosen because they formed in different climatic locations, were from diverse areas of the Great Plains and eastern Rocky Mountains, or handled by different agencies.

Seeds underwent 16 weeks of stratification in sand prior to the testing procedures. This stratification consisted of a 6 week warm-moist period (24^0C) followed by a 10 week

cool-moist period (5°C) as suggested by Van Haverbeke and Comer (1985). After the various germination treatments, the seeds were placed in Petri dishes on a filter substratum before being placed in a Stults germinator (model SD-48) at 15°C. The germinator was set for 8 hours of light and 16 hours of dark as determined by Van Haverbeke and Comer (1985). Replications one and two from each treatment were placed in the left side of the germinator while replications three and four were placed on the right side of the germinator. When fungal growth was present in the Petri dishes, fungicide (50W Captan) was applied.

Counts were recorded for germination and the number of seeds with broken seed coats over a four month period. Germination (G), which was considered a 1 mm protrusion of the radicle, was counted every other day during the four month period. At the end of the four month period, seeds were checked to determine if the seed coat had cracked (B) (seed coat dormancy broken). All seeds were then discarded. G+B is the number of seeds that germinated plus the number of seeds with broken seed coats. G+B[a] is the number of seeds that germinated plus the number of seeds with broken seed coats given as a function of the initial viability for each seed lot.

Seeds were tested for viability using TZ before and after germination trials to determine if the treatments adversely affected the viability of the seeds (International Seed Testing Association 1985). The TZ used for viability testing was 2, 3, 5-Triphenyl-2H-Tetrazolium Chloride (FW 334.81).

3.2.2. Experimental treatments

3.2.2.1. Gibberellic acid (GA_3)

Seeds from five seed lots (ERC-1, ERC-2, RMJ-1, RMJ-2, and RMJ-3) were treated with three concentrations of GA_3 (FW 384.5). The three concentrations of GA_3 were 48, 130, and 216 x 10^{-5}M as determined by Singh (1989). Prior to placing the seeds in the germinator, the substratum that the seeds were placed onto was saturated with the different solutions of gibberellic acid. Four replications containing 25 seeds per replication were treated with each of the three solutions. This procedure was repeated for all five seed lots. Viability testing was conducted on seeds exposed to the 130 and 216 x 10^{-5}M solutions.

3.2.2.2. Potassium nitrate (KNO_3)

Seeds from five seed lots (ERC-1, ERC-2, RMJ-1, RMJ-2, and RMJ-3) were treated with KNO_3 (FW 141.96). The level of KNO_3 used was a 0.2% solution (Association of Official Seed Analysts 1994). Prior to placing the seeds in the germinator, the substratum that the seeds were placed onto was saturated with a 0.2% solution of KNO_3. Four replications of 25 seeds per seed lot were used for the KNO_3 treatment. Viability testing was conducted on all seeds that were treated with KNO_3.

3.2.2.3. Osmotic priming (PEG)

Seeds from five seed lots (ERC-1, ERC-2, RMJ-1, RMJ-2, and RMJ-3) were osmotically primed using three solutions of PEG (MW 10,000). The three osmotic potentials were -0.5, -1.0, and -1.5 MPa. The three solutions were placed in 1.5" diameter plastic tubes and aerated from the bottom of the tube. Four replications of 25 seeds per

replication were treated with each of the three solutions. This procedure was repeated for all five seed lots. The seeds were primed for 11 days. The solution was changed after 8 days due to hardening of the solution. Prior to placing the seeds in the germinator, the substratum that the seeds were placed onto for germination trials was saturated with distilled water. Seeds primed at -1.5 MPa were tested for viability after priming.

3.2.2.4. Rock polisher

Seeds from all eight seed lots were used for the rock polisher experiment. A Thumblers rock polisher (Model A-R2 No. 115) was used to tumble the seeds for 0, 1, 2, 3, or 6 hour durations. The tumbling was performed to soften or break down the seed coat. Course grit (Carborundum) was used during the tumbling of the seeds. Four replications containing 25 seeds per replication were treated with each of the five different durations of tumbling. This procedure was repeated for all eight seed lots. Prior to placing the seeds in the germinator, the substratum that the seeds were placed onto for germination trials was saturated with distilled water. Viability was tested on seeds tumbled for 0 and 6 hours.

3.2.2.5. Size-density separation

Seeds from five seed lots were used in this experiment. They were ERC-1, ERC-2, RMJ-1, RMJ-2, and RMJ-3. Three hundred seeds from each seed lot were separated into 3 size classes which were further separated into 3 density classes, resulting in a total of 9 groups. Size separation was done by shaking the seeds through soil sieves. Each size class was then separated into three density classes using sodium chloride solutions. For ERC, the three size classes were >2.44 mm, 2.44-2.29 mm, and <2.29 mm. The three

density classes for ERC were >1.224 g/cm^3, 1.224-1.200 g/cm^3, and <1.200 g/cm^3. For RMJ, the three size classes were >3.15 mm, 3.15-2.46 mm, and <2.46 mm. The three density classes for RMJ were >1.224 g/cm^3, 1.224-1.200 g/cm^3, and <1.200 g/cm^3. Prior to placing the seeds in the germinator, the substratum that the seeds were placed onto for germination trials was saturated with distilled water. All seeds were tested for viability following germination trials.

3.2.2.6. Incubation dehydration separation (IDS)

Seeds from five seed lots (ERC-1, ERC-2, RMJ-1, RMJ-2, and RMJ-3) were separated using the Incubation, Dehydration, Separation (IDS) technique. Four replications containing 25 seeds per replication were separated with this technique. The seeds were incubated for 72 hours at 21^{0}C on filter paper moistened with distilled water. Seeds then underwent a 36 hour desiccation period at 23% RH and 21^{0}C. Seeds were then separated in tap water as either sinkers or floaters. Overall viability was calculated as an average of the floaters and sinkers for each seed lot.

3.2.2.7. Leachate conductivity

Seeds from five seed lots (ERC-1, ERC-2, RMJ-1, RMJ-2, and RMJ-3) were examined to determine their leachate conductivities. One gram of seeds from each seed lot was placed in a test tube with 10ml of deionized distilled water. The conductivity of the water was measured to correct for the initial conductance. The test tubes were placed on a rotating plate at 100 RPM and soaked for five hours. Conductivity was measured with a YSI (Model 35) conductance meter. After five hours of soaking, the leachate conductivity was measured again. Each test tube was then microwaved for approximately

30 seconds or until the water started to boil. After microwaving, leachate conductivity was measured once again. The percent of maximum leakage (Leakage Index) was obtained by dividing the 5 hour conductivity by the microwaved conductivity.

3.2.2.8. Control

The same control was used for all the treatments to break dormancy and the size and density separation technique. This was due to all the experiments being conducted at the same time and in the same germination cabinet. The control seeds were stratified along with all the treated seeds. When the stratification period was complete, the control seeds were placed on filter paper moistened with distilled water and put in the germinator along with the treated seeds. As with all the seeds in the germinator, the filter papers were moistened with distilled water again when they began to dry out.

3.2.3. Statistical analysis

Analysis of variance (ANOVA) of the treatment means for percent germination, broken seed coats, and G+B was calculated using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC 1993). All tests were conducted using a randomized complete block design with the exception of the size-density separation test. The separation test was conducted as a completely randomized design. Transformation of the data using $x' = \sqrt{(x+1)} + \sqrt{(x)}$ was justified by SAS Univariate analysis. Where significant treatment or seed lot effects were indicated, means were separated by Tukey's studentized range test at $\alpha = 0.05$.

3.3. Results and discussion

Overall, despite the many treatments used in this study, germination remained extremely low (Appendix 1). As would be expected, overall germination was higher for eastern redcedar when compared to Rocky Mountain juniper (Figure 1, Appendix 2). Results by individual treatments are discussed separately below.

3.3.1. Gibberellic acid

Treatment with GA_3 significantly increased germination as compared to the control (Figure 2, Appendix 1). However, treatment with GA_3 did not significantly increase the number of seed with broken seed coats as compared to the control (Figure 2, Appendix 1). Germination percent, broken seed coats, and G+B over all seed lots were significantly higher for ERC-2 than for the other seed lots tested (6.7, 7.7, and 14.3% respectively) (Figure 3). There were no significant differences among the GA_3 concentrations with respect to germination percent, broken seed coats, and G+B (Table 2). The 130 x 10^{-5}M solution consistently increased germination percent, number of seed with broken seed coats, and G+B as compared to the 48 and 216 x 10^{-5}M solutions, however these differences were not significant at the 0.05 level (Appendix 3). Percent G+B is given as a function of viability in Appendix 3.

Geneve (1991) found that while GA_3 increased germination rates of Cercis canadensis (var. canadensis L.) over the controls, the germination rates by solution were not significantly different. Similarly, solution concentration did not have a significant effect on germination in this study. These results are in contrast with the results of Persson (1993) and Durrant and Mash (1991) who found that significant differences in

germination were apparent as the concentration of GA_3 was altered. A possible explanation for the lack of differences in germination rates in our study over different concentrations of GA_3 is that hormones are active in very small amounts. If the hormonal balance has been shifted to promote germination at low concentrations, applying more GA_3 should not increase the germination rate further.

3.3.2. Potassium nitrate

Treatment with KNO_3 did not significantly increase percent germination, the number of seed with broken seed coats, or G+B as compared to the control (Appendix 4). The highest germination percentage was 1% for ERC-1 and ERC-2 (Appendix 5). The highest G+B percentage was 7% for ERC-2. Percent G+B is given as a function of viability in Appendix 5.

For this experiment, KNO_3 did not significantly increase percent germination and G+B over the control seeds. In fact, KNO_3 seemed to have a detrimental effect on the seeds and actually lowered the germination and G+B percentage as compared to the control (Appendix 1).

3.3.3. Osmotic priming

Osmotic priming with PEG did not significantly increase percent germination, the number of seed with broken seed coats, or G+B as compared to the control (Figure 2, Appendix 1). However, significant differences were found for concentration and seed lot x concentration with respect to percent germination and G+B (Table 3). No significant differences were found for concentration and seed lot x concentration for broken seed coats (Table 3). The highest percent germination by solution was from the -0.5 MPa

solution at 1% (Appendix 6). The highest percent G+B by solution was from the -1.0 MPa solution at 1.6% (Appendix 6). Percent G+B is given as a function of viability in Appendix 6.

For this study, osmotic priming did not significantly increase percent germination, broken seed coats, or G+B as compared to the control seeds. Restricting water availability to the seeds could actually cause the seeds to become more dormant. As the data shows (Appendix 1), osmotic priming actually reduced the percent germination, broken seed coats, and G+B. This decrease was not significant at the 0.05 level. Also, the lower concentrations of PEG led to higher germination percentages. This suggests that the PEG had a detrimental effect on the seeds with increased concentrations. Since the highest germination percent was from the highest water potential, it seems that the lower the osmotic potential, the more harmful the treatment may be to the Juniperus seeds.

3.3.4. Rock polisher

The treatment of tumbling seeds in a rock polisher did not significantly increase percent germination, the number of seed with broken seed coats, or G+B as compared to the control (Figure 2, Appendix 1). Significant differences were found for seed lot with respect to G+B but not for duration with respect to G+B (Appendix 7). The highest percentages for germination, broken seed coats, and G+B (1.5, 5.1, and 6.6% respectively) were from the three hour tumble (Appendix 8). ERC-3 with a six hour tumble had the highest percent for G+B (27%) (Figure 4). Percent G+B is given as a function of viability in Appendix 8.

Contrary to Holthuijzen and Sharik's (1985) findings for bird passed seeds, tumbling seeds did not increase germination as compared to the control. A possible explanation for the lack of a significant increase in germination is that no chemical scarification was performed on the rock tumbled seeds. The gizzard of a bird provides both mechanical (churning) and chemical (gastric acid) scarification of the seeds. This study also shows that the duration of tumble had no effect on the germination rates of the seeds. Although seeds pass unharmed through the digestive tract of birds in only thirty minutes (Holthuijzen and Sharik 1985), the six hour tumble in our study seemed to be too short of a tumble. After six hours, physical damage to the seed coat could not be detected.

3.3.5. Size-density separation

Separating seeds by size and density significantly increased percent germination, the number of seed with broken seed coats, and G+B over all size and density classes as compared to the control (Figure 2, Appendix 1). These results were unexpected because it implies that something in the separation process (shaking causing possible abrasion of the seed coat with the subsequent soaking in a salt solution) altered dormancy. No significant differences were found for size, density, or size x density with respect to percent germination (Appendix 9). The highest percent germination for the size separation technique was 5.4% for the small seeds (Table 4). This seed size also happened to be the most viable over all seed sizes (Figure 5). The highest percent germination for the density separation technique was 4.7% for the medium density seeds (Table 4). Significant differences were found for size, density, and size x density with respect to G+B

(Figure 6). Percent G+B was significantly higher for the small sized seeds (16.9%) as compared to the large sized seeds (4.6%) (Table 4). Percent G+B was significantly higher for the medium density seeds (14.5%) as compared to the low density seeds (7.2%) (Table 4). Percent G+B is given as a function of viability in Table 4.

Separation of seeds by size and density has proven to be an effective method for increasing seed germination in other species. Khademi et al. (1993) demonstrated that as the density of Primula acaulis (L) seed rose, the percent viability increased. Similarly, medium and heavy seeds germinated at a higher percentage than the light seeds in the present study. This is likely due to the light seeds being empty or having a damaged or immature embryo.

In this study, the small seeds had the highest percent germination. This coincides with the findings of Reich et al. (1994) who found that small seeds of Scots pine (Pinus sylvestris L.) exhibited the highest percent germination. A possible explanation for small seeds having the highest percent germination in eastern redcedar and Rocky Mountain juniper is that their seed coats are thinner than the seed coats of the larger seeds. This would allow the smaller seeds to germinate at a higher percentage as compared to the larger seeds because the embryo of the small seeds would have to exert less pressure to germinate. Thinner seed coats would also allow for increased water and oxygen movement into and out of the seed which could increase respiration and water uptake and thus increase germination.

3.3.6. Incubation, dehydration, separation

Significant differences were detected by seed lot with respect to the viability of the sinkers but not for the floaters. Viability of the sinkers was significantly higher than the viability of the floaters (71 and 22%, respectively) (Figure 7). There was no significant difference in the viability of the sinkers and the overall viability across all seed lots (Appendix 10). However, in all cases the viability of the sinkers was higher than the overall viability. In this study, ERC-1 sinkers produced the highest viability of 83%. The lowest viability was 8% from RMJ-2 floaters (Appendix 11). Overall, viability was higher for eastern redcedar seed lots than Rocky Mountain juniper seed lots.

The IDS technique is based on the principle that viable seeds, once hydrated, release moisture more slowly than unfilled or non-viable seeds. Vozzo and Singh (1994) and Bergsten (1988) reported that germination was significantly increased using the IDS technique for Pinus roxburghii (Sarg.) and Pinus sylvestris (L.) seeds respectively. In a study of lodgepole pine (Pinus contorta Dougl.), Simak (1984) found that almost all seeds that did not germinate were found to be dead using the IDS technique. While germination was not tested for this treatment, we found that significant differences were apparent for viability of seeds that sank as compared to seeds that floated (Figure 7). Seeds that sink possibly have a complete, mature embryo where floaters may have immature or absent embryos. By using the IDS technique of seed separation, more viable seed will be sown possibly resulting in increased germination rates.

3.3.7. Leachate conductivity

Significant differences were detected for the leachate conductivity at 5 hours of soaking and after microwaving as compared by seed lot. Significant differences were also found for leakage index by seed lots (Appendix 12). Leakage indices were higher for Rocky Mountain juniper as compared to eastern redcedar seed lots, indicating greater leakage of electrolytes for Rocky Mountain juniper seeds (Figure 8). This coincides with the initial TZ viability data for these seed lots.

Leachate conductivity relies on the principle that as seed quality decreases, the amount of solutes leached from the seeds increases. Vozzo (1994) and Bonner and Vozzo (1986) found that leachate conductivity can be a useful tool to predict laboratory germination of loblolly pine (Pinus taeda L.). By using leachate conductivity data to determine seed lot viability, eastern redcedar seed lots were found to be more viable than the Rocky Mountain juniper seed lots. The leachate conductivity data support the TZ data that the eastern redcedar seed lots were generally more viable than the Rocky Mountain juniper seed lots. Leachate conductivity is a method which shows promise for predicting the quality of eastern redcedar and Rocky Mountain juniper seed lots.

3.4. Conclusion/Management Implications

In the attempt to increase germination with methods that break seed dormancy, it was found that applications of gibberellic acid (GA_3) was the only treatment tested which would significantly increase germination. Having said this, it is also important to note that this increase in germination remained too low to be of value for good nursery production. This study shows that a treatment used to break seed coat or embryo dormancy alone will

not increase germination rates to acceptable levels. The results suggest that if you are to increase germination in seeds of eastern redcedar and Rocky Mountain juniper, a method must be developed to break seed coat and embryo dormancy in that order as is the case with seeds passing through the gizzard of a bird. You must break the seed coat dormancy in order for the embryo treatment to reach the embryo where it is to perform.

The separation techniques were examined for their ability to select out seed populations with higher viabilities. The separation of seed by size and density worked well to accomplish this task. The small sized high density seeds had the highest overall germination while exhibiting the highest viabilities when compared to the large sized low density seeds. The IDS technique also helped to separate out a more viable subsample of seeds although the increase in viability was not significant.

Leachate conductivity was found to be a useful tool for evaluating the initial quality for a seed lot of eastern redcedar or Rocky Mountain juniper. Leachate conductivity was found to correlate well with TZ viability analysis ($r = .95$, $P < 0.02$). In general, the eastern redcedar seed lots were found to be more viable than the Rocky Mountain juniper seed lots. This would help explain the increased germination results for eastern redcedar when compared to the germination results of Rocky Mountain juniper. The leachate conductivity results are an important finding because it is an easily performed method of assessing the initial seed quality as compared to the more time consuming and tedious task of determining viability using the TZ method.

The next logical step would be to combine a treatment to break seed coat dormancy followed by a treatment to break embryo dormancy. This could be a tumble in

the rock polisher followed by an application of GA_3. In addition, nursery managers should find the separation and leachate conductivity results important. By sowing more viable seeds, a nursery manager should be able to increase the number of seeds that germinate as well as increase the survival rate of the seedlings with minimal effort. Leachate conductivity would also be valuable in determining the number of seeds to be sown in order to achieve an acceptable crop of eastern redcedar and Rocky Mountain juniper. This would help save much needed bed space in the nursery. By using these results, nursery managers will be able to better supply the increasing demand for Juniperus seedlings in the Great Plains now and into the future.

3.5. References

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Table 1. Location, date of collection, and collecting agency of the seed lots used in the study.

Seed lot	Location	Collection Date	Collected by
ERC-1*	Washington, OK	1993	ODF[a]
ERC-2*	Washington, OK	1993	ODF[a]
ERC-3	Anselmo-Merna, NE	1987	USFS[b]
RMJ-1	Saguaches, CO	1992	CFS[c]
RMJ-2	Crestone, CO	1988	CFS[c]
RMJ-3**	Wasta, SD	1988	USFS[b]
RMJ-4	Creighton-Wall, SD	1983	SDFS[d]
RMJ-5**	Wasta, SD	1988	USFS[b]

[a]Forest Regeneration Center, Oklahoma Division of Forestry.
[b]Unites States Forest Service, Bessey Nursery.
[c]Colorado Forest Service, Colorado State Nursery.
[d]South Dakota Forest Service, Big Sioux Nursery.
*ERC-1 was dried and then frozen. ERC-2 was dried and then stratified.
**RMJ-3 and RMJ-5 are different seed sources.

Table 2. Analysis of variance for germination (G), broken seed coats (B), and germination + broken seed coats (G+B) for seeds treated with gibberellic acid.

Source of		G		B		G+B	
variation[a]	df	MS	F	MS	F	MS	F
S	1	0	0.01^{ns}	0.022	1.78^{ns}	0.007	0.55^{ns}
R(S)	2	0.001	0.19^{ns}	0.003	0.25^{ns}	0.005	0.37^{ns}
SL	4	0.168	26.68*	0.167	13.74*	0.385	31.25*
SO	2	0.016	2.47^{ns}	0.013	1.03^{ns}	0.013	1.04^{ns}
SO x SL	8	0.011	1.74^{ns}	0.013	1.06^{ns}	0.014	1.16^{ns}
Error	42	0.006		0.012		0.012	

NOTE: significance levels are given as probability: ^{ns}, $P>0.05$ and *, $P<0.05$.
[a]S, side; R(S), rep(side); SL, seed lot; SO, solution.

Table 3. Analysis of variance for germination (G), broken seed coats (B), and germination + broken seed coats (G+B) for seeds treated with polyethylene glycol.

Source of		G		B		G+B	
variation[a]	df	MS	F	MS	F	MS	F
S	1	0.002	0.53^{ns}	0.01	1.88^{ns}	0.001	0.13^{ns}
R(S)	2	0.006	1.75^{ns}	0.005	0.96^{ns}	0.018	3.43*
SL	4	0.018	5.48*	0.017	3.30*	0.06	11.46*
C	2	0.013	3.83*	0.008	1.49^{ns}	0.024	4.65*
SL x C	8	0.013	3.90*	0.004	0.72^{ns}	0.013	2.42*
Error	42	0.003		0.005		0.005	

NOTE: significance levels are given as probability: ^{ns}, P>0.05 and *, P<0.05.
[a]S, side; R(S), rep(side); SL, seed lot; C, concentration.

Table 4. Effect of separation by size and density on percent germination (G), broken seed coats (B), germination + broken seed coats (G+B), and germination + broken seed coats as a function of viability (G+B[a]) by seed lot.

		G	B	G+B	G+B[a]
Seed lot	ERC-1	1.2b	3.5bc	4.7b	6.4b
	ERC-2	14.9a	9.9ab	24.9a	31.1a
	RMJ-1	0.2b	0.4c	0.6b	0.8b
	RMJ-2	1.1b	3.3c	4.4b	6.4b
	RMJ-3	3.5b	15.7a	19.2a	30.0a
Size	Small	5.4a	11.5a	16.9a	23.7a
	Medium	4.2a	6.6a	10.8ab	15.1ab
	Large	3.0a	1.7b	4.6b	6.0b
Density	Low	3.6a	3.8b	7.2b	9.9a
	Medium	4.7a	9.8a	14.5a	20.5a
	High	4.5a	6.1ab	10.6ab	14.5a

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$) with respect to transformed data.

Figure 1. Effects of all treatments on the percentage of germination (G), broken seed coats (B), and germination + broken seed coats (G+B) in eight different seed lots. G+B is the sum of germination and broken seed coats. The vertical lines on the bar graph represent the standard error.

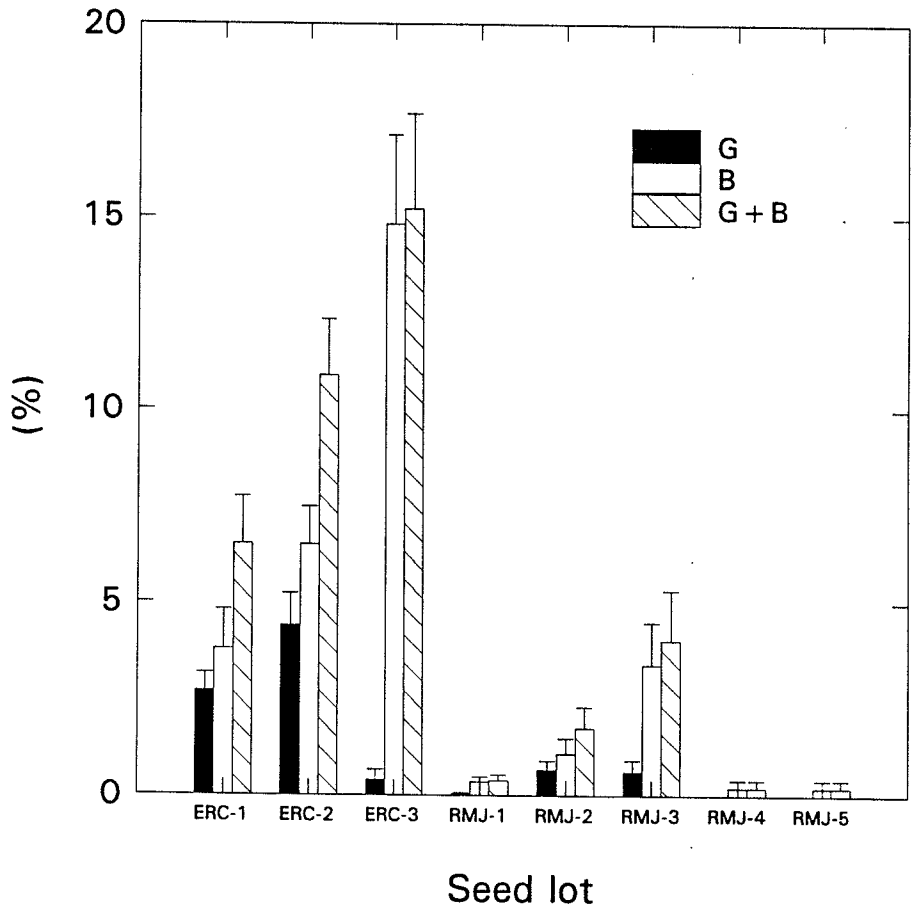

20
15
10
5
0
(%)
G
B
G + B
ERC-1
ERC-2
ERC-3
RMJ-1
RMJ-2
RMJ-3
RMJ-4
RMJ-5
Seed lot

Figure 2. Effects of all treatments on the percentage of germination (G), broken seed coats (B), and germination + broken seed coats (G+B) averaged over all treatments. The vertical lines on the bar graph represent the standard error.

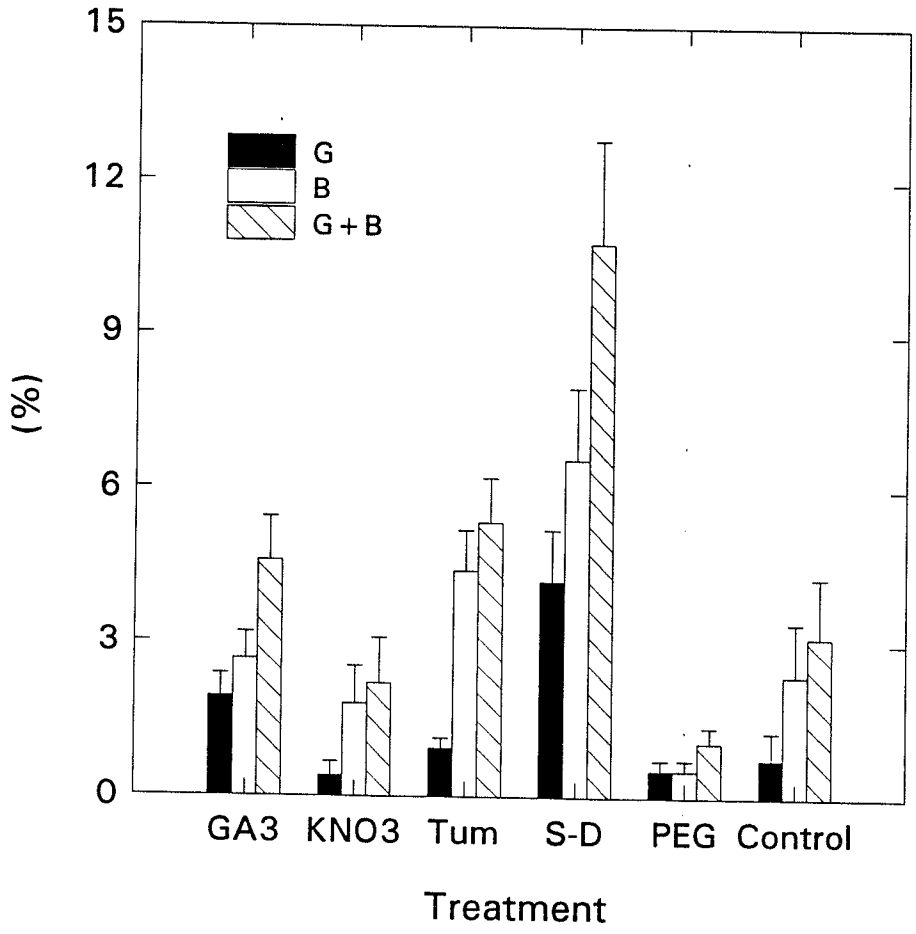

15
12
9
6
3
0
(%)
G
B
G+B
GA3
KNO3
Tum
S-D
PEG
Control
Treatment

Figure 3. Effects of the concentration of gibberellic acid solution on the percentage of germination + broken seed coats (G+B) in five different seed lots. G+B is the number of seeds that germinated plus the number of seeds with broken seed coats at the end of the germination trials. The three solutions were $48x10^{-5}M$, $130x10^{-5}M$, and $216x10^{-5}M$. The vertical lines on the bar graph represent the standard error.

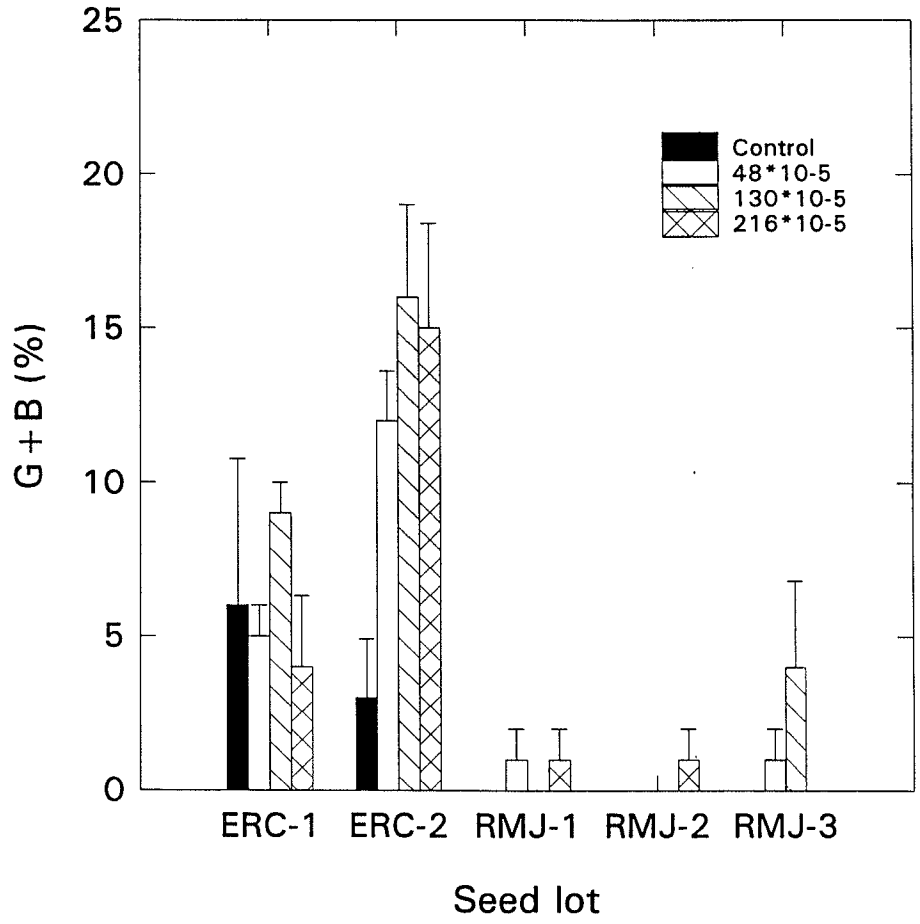

25
20
15
10
5
0
G+B (%)
Control
48*10-5
130*10-5
216*10-5
ERC-1
ERC-2
RMJ-1
RMJ-2
RMJ-3
Seed lot

Figure 4. Effects of the duration of time in the rock polisher on the percentage of germination + broken seed coats (G+B) in eight different seed lots. G+B is the number of seeds that germinated plus the number of seeds with broken seed coats at the end of the germination trials. The five tumbling durations were 0, 1, 2, 3, and 6 hours. The vertical lines on the bar graph represent the standard error.

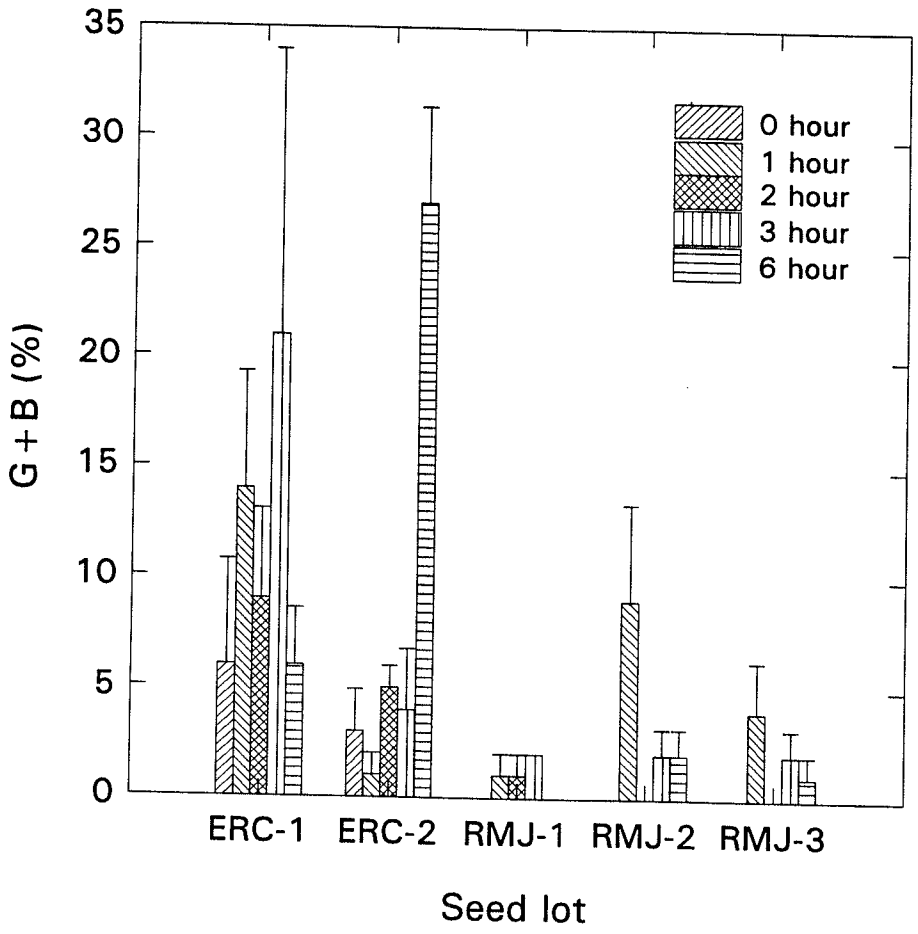

35
30
25
20
15
10
5
0
G+B (%)
0 hour
1 hour
2 hour
3 hour
6 hour
ERC-1
ERC-2
RMJ-1
RMJ-2
RMJ-3
Seed lot

Figure 5. Effects of separation by size and density on the viability averaged over five different seed lots. Nine separate predetermined classes were created: three size classes and three density classes.

Viability Percentage

by Size and Density

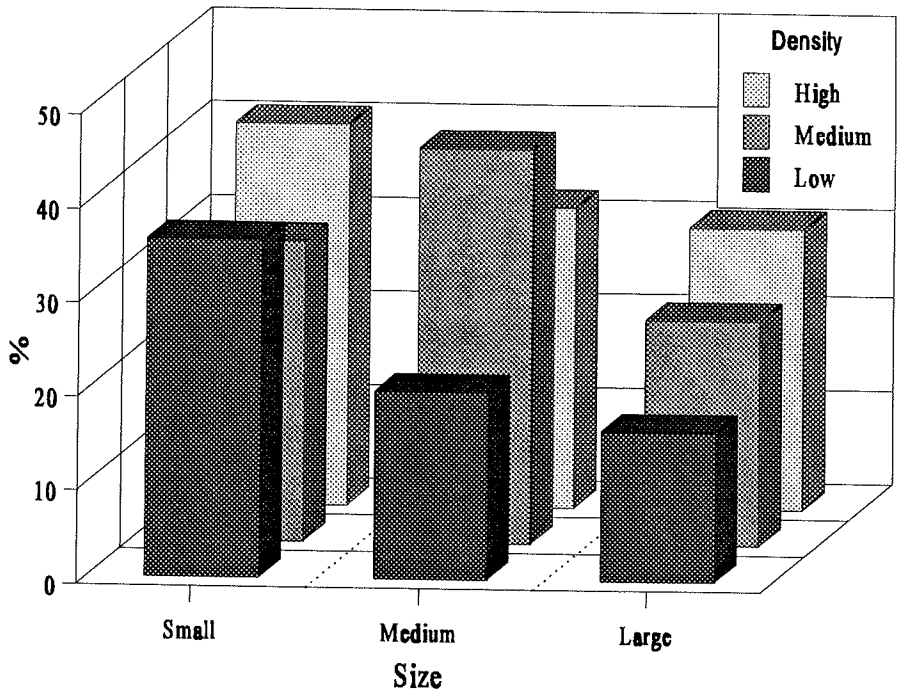


Figure 6. Effects of seed size and density on the percentage of germination + broken seed coats (G+B) in five different seed lots. G+B is the number of seeds that germinated plus the number of seeds with broken seed coats at the end of the germination trials. Nine separate predetermined classes were created: three size classes and three density classes.

G+B Percentage

by Size and Density

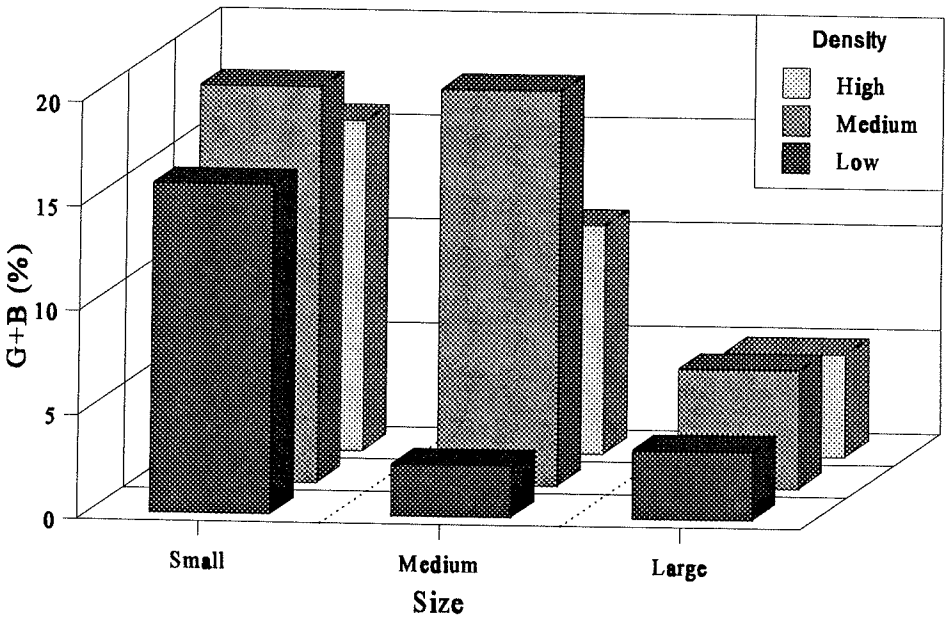


Figure 7. Effects of seed separation by the Incubation, Dehydration, Separation (IDS) technique on the viability in five different seed lots. The vertical lines on the bar graph represent the standard error.

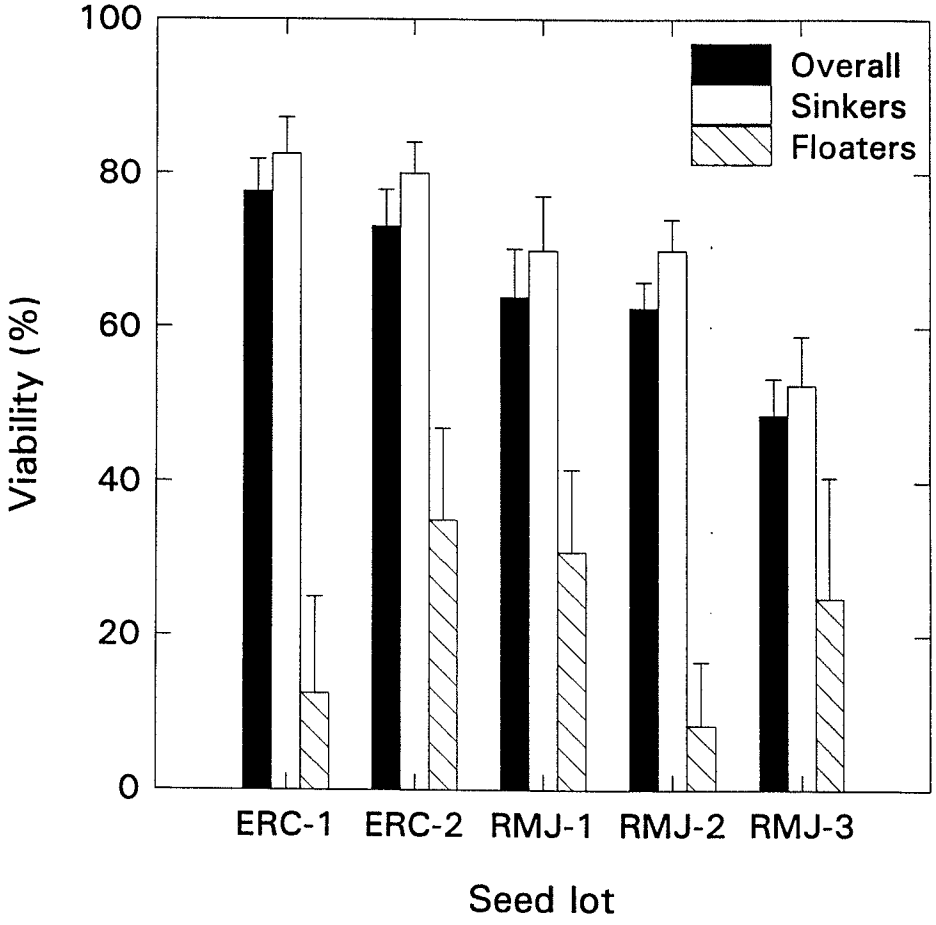
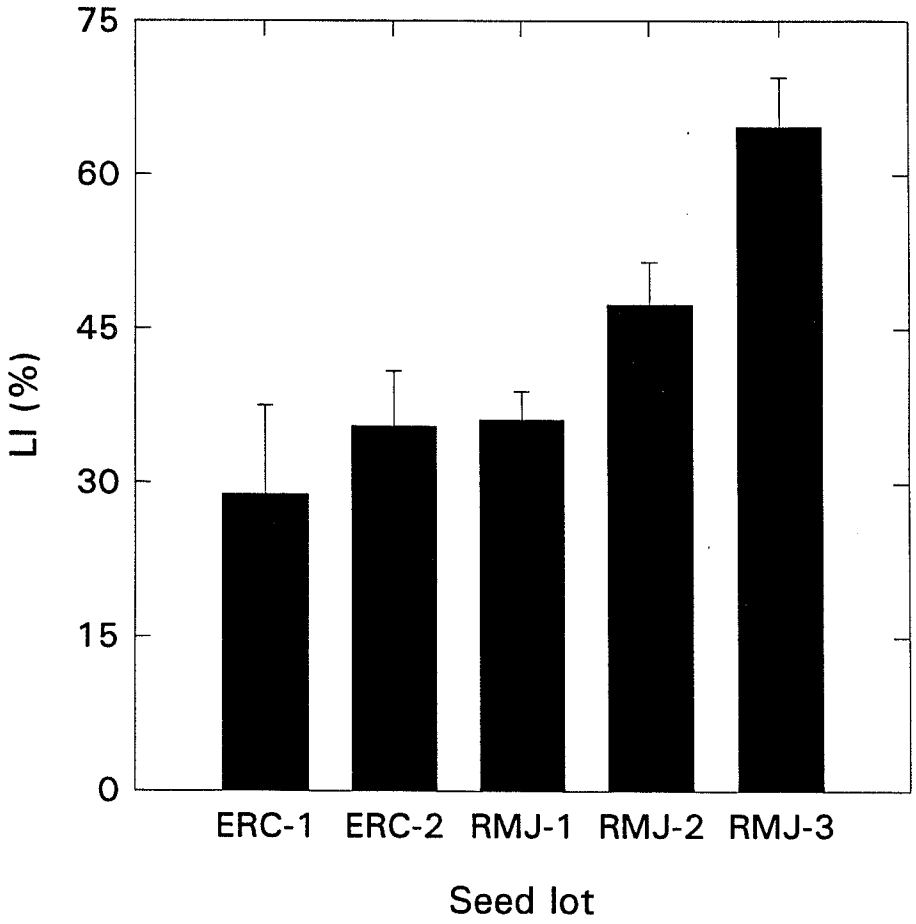

100
80
60
40
20
0
Viability (%)
Overall
Sinkers
Floaters
ERC-1
ERC-2
RMJ-1
RMJ-2
RMJ-3
Seed lot

Figure 8. Leakage index (LI) in five different seed lots. LI is the leachate conductivity of the seed lot at five hours divided by the total leachate conductivity of the seed lot. The vertical lines on the bar graph represent the standard error.


75
60
45
30
15
0
LI (%)
ERC-1
ERC-2
RMJ-1
RMJ-2
RMJ-3
Seed lot

CHAPTER 4

SUMMARY AND CONCLUSIONS

This study examined the effectiveness of various treatments and separation techniques on germination and viability in seeds of eastern redcedar and Rocky Mountain juniper. Three areas were identified as promising avenues to enhance production of eastern redcedar and Rocky Mountain juniper. These three areas consisted of methods to break seed dormancy (gibberellic acid (GA_3), potassium nitrate (KNO_3), osmotic priming (PEG), and tumbling seeds in a rock polisher), seed separation to receive a more viable subsample of seeds (size-density and Incubation, Dehydration, Separation (IDS)), and a method to quickly assess the initial viability of a seed lot (leachate conductivity (LC)).

Of the four treatments used to break seed dormancy, the only treatment to significantly increase germination was the application of GA_3 to the seeds. It is important to note that this increase remained too low to be of value to a nursery manager (from 0.8% for control to 1.9% for applications of GA_3). As would be expected, overall germination of eastern redcedar was higher than that of Rocky Mountain juniper. This study shows that a treatment used to break seed coat or embryo dormancy alone was not successful in enhancing germination to production levels and that a combination of treatments for both seed coat and embryo dormancy is needed.

In the attempt to separate out a more viable subsample of seeds for planting, the seed separation by size and density worked well. The small sized high density seeds exhibited higher viabilities when compared to the large sized low density seeds. This

method also significantly increased germination across all size and density classes as compared to the control treatment (from 0.8% for control to 4.2% for size and density separation). This indicates something in the technique reduced dormancy, most likely seed coat dormancy during the size separations. The IDS technique also helped to separate out a more viable subsample of seeds for planting although the increase in viability was not significant.

Leachate conductivity was found to be a useful tool for evaluating the initial quality for a seed lot of eastern redcedar or Rocky Mountain juniper. Leachate conductivity was found to correlate well with the tetrazolium chloride (TZ) viability analysis ($r = .95$, $P < 0.02$). In general, the eastern redcedar seed lots were found to be more viable than the Rocky Mountain juniper seed lots. This would help explain the increased germination results for eastern redcedar when compared to the germination results of Rocky Mountain juniper. The leachate conductivity results are an important finding because it is an easily performed method of assessing the initial seed quality as compared to the more time consuming and tedious task of determining viability using the TZ method.

A limitation of this study which may be an explanation for the relatively low germination rates is that all germination tests were conducted in a germinator rather than the field. Beneficial effects of the environment such as temperature fluctuations and soil microbial action are absent in the germinator and it was not feasible in this study to sow the large quantity of seeds tested.

The next logical step would be to combine a treatment to break seed coat

dormancy followed by a treatment to break embryo dormancy. This could be a tumble in the rock polisher followed by an application of GA_3. In addition, nursery managers should find the separation and leachate conductivity results important. By sowing more viable seeds, a nursery manager should be able to increase the number of seeds that germinate as well as increase the survival rate of the seedlings with minimal effort. Leachate conductivity would also be valuable in determining the number of seeds to be sown in order to achieve an acceptable crop of eastern redcedar and Rocky Mountain juniper. This would in turn help nursery managers save much needed bed space in the nursery. By using these results, nursery managers will be able to better supply the increasing demand for Juniperus seedlings in the Great Plains now and into the future.

Appendix 1. Effect of all treatments compared to the control for percent germination (G), broken seed coats (B), germination + broken seed coats (G+B), and germination + broken seed coats as a function of viability (G+B[a]) by the seed lot and the treatment.

		G	B	G+B	G+B[a]
Seed lot	ERC-1	2.7a	3.8bc	6.5bc	8.9bc
	ERC-2	4.4a	6.5b	10.9ab	13.6ab
	ERC-3	0.4b	14.8a	15.2a	19.2a
	RMJ-1	0.0b	0.3d	0.4d	0.5de
	RMJ-2	0.7b	1.1cd	1.8d	2.5de
	RMJ-3	0.6b	3.4cd	4.0cd	6.3cd
	RMJ-4	0.0b	0.2d	0.2d	0.2
	RMJ-5	0.0b	0.2d	0.2d	0.3
Treatment	Control	0.8c	2.4bc	3.1bc	4.0bc
	GA_3	1.9b	2.7bc	4.6b	6.0b
	KNO_3	0.4c	1.8bc	2.2bc	2.9bc
	PEG	0.5c	0.5c	1.1c	1.4c
	Separation	4.2a	6.6a	10.8a	14.9a
	Tumble	0.9bc	4.4ab	5.3b	7.0b

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$) with respect to transformed data.

Appendix 2. Analysis of variance for germination (G), broken seed coats (B), and germination + broken seed coats (G+B) as compared to all treatments.

Source of		G		B		G+B	
variation[a]	df	MS	F	MS	F	MS	F
SL	7	0.12	15.54*	0.395	19.34*	0.555	23.81*
T	5	0.09	11.71*	0.187	9.16*	0.324	13.91*
T x SL	23	0.06	7.48*	0.042	2.08*	0.083	3.56*
Error	309	0.01		0.02		0.023	

NOTE: significance levels are given as probability: ^{ns}, P>0.05 and *, P<0.05.
[a]SL, seed lot; T, treatment.

Appendix 3. Effect of gibberellic acid on percent germination (G), broken seed coats (B), germination + broken seed coats (G+B), and germination + broken seed coats as a function of viability ($G+B^a$) by the seed lot and the concentration of gibberellic acid solution.

		G	B	G+B	$G+B^a$
Seed lot	ERC-1	2.7b	3.3b	6.0b	8.2b
	ERC-2	6.7a	7.7a	14.3a	17.9a
	RMJ-1	0.0c	0.7b	0.7c	0.8c
	RMJ-2	0.3bc	0.0b	0.3c	0.5c
	RMJ-3	0.0c	1.7b	1.7bc	2.6bc
Solution	Control	0.8b	2.4a	3.1b	4.0a
	$48x10^{-5}$	1.0ab	2.8a	3.8ab	4.9a
	$130x10^{-5}$	2.6a	3.2a	5.8a	7.7a
	$216x10^{-5}$	2.2ab	2.0a	4.2ab	5.4a

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$) with respect to transformed data.

Appendix 4. Analysis of variance for germination (G), broken seed coats (B), and germination + broken seed coats (G+B) for seeds treated with potassium nitrate.

Source of		G		B		G+B	
variation[a]	df	MS	F	MS	F	MS	F
S	1	0.006	0.77^{ns}	0.002	0.24^{ns}	0	0.00^{ns}
R(S)	2	0.026	3.50*	0.007	0.79^{ns}	0.037	2.16^{ns}
SL	4	0.021	2.82*	0.037	3.96*	0.05	2.91*
T	1	0.006	0.77^{ns}	0.022	2.4^{ns}	0.009	0.52^{ns}
Error	31	0.007		0.009		0.017	

NOTE: significance levels are given as probability: ^{ns}, P>0.05 and *, P<0.05.
[a]S, side; R(S), rep(side); SL, seed lot; T, treatment.

Appendix 5. Effect of potassium nitrate on percent germination (G), broken seed coats (B), germination + broken seed coats (G+B), and germination + broken seed coats as a function of viability ($G+B^a$) by seed lot.

		G	B	G+B	$G+B^a$
Seed lot	ERC-1	1.0a	0.0a	1.0a	1.4a
	ERC-2	1.0a	6.0a	7.0a	8.8a
	RMJ-1	0.0a	0.0a	0.0a	0.0a
	RMJ-2	0.0a	1.0a	1.0a	1.5a
	RMJ-3	0.0a	2.0a	2.0a	3.1a

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$) with respect to transformed data.

Appendix 6. Effect of polyethylene glycol on percent germination (G), broken seed coats (B), germination + broken seed coats (G+B), and germination + broken seed coats as a function of viability (G+B[a]) by seed lot and concentration of polyethylene glycol.

		G	B	G+B	G+B[a]
Seed lot	ERC-1	1.0ab	1.3a	2.3a	3.2a
	ERC-2	1.7a	1.3a	3.0a	3.8a
	RMJ-1	0.0b	0.0a	0.0b	0.0b
	RMJ-2	0.0b	0.0a	0.0b	0.0b
	RMJ-3	0.0b	0.0a	0.0b	0.0b
Solution	-0.5 MPa	1.0a	0.4a	1.4ab	1.8ab
	-1.0 MPa	0.6ab	1.0a	1.6a	2.1a
	-1.5 MPa	0.0b	0.2a	0.2b	0.3b

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$) with respect to transformed data.

Appendix 7. Analysis of variance for germination (G), broken seed coats (B), and germination + broken seed coats (G+B) for seeds treated with the rock polisher.

Source of		G		B		G+B	
variation[a]	df	MS	F	MS	F	MS	F
S	1	0.003	0.50^{ns}	0.03	1.43^{ns}	0.027	1.28^{ns}
R(S)	2	0.005	0.93^{ns}	0.023	1.11^{ns}	0.042	1.99^{ns}
SL	7	0.093	17.00*	0.421	20.36*	0.51	24.21*
D	4	0.016	2.97*	0.022	1.07^{ns}	0.039	1.85^{ns}
SL x D	28	0.007	1.31^{ns}	0.047	2.27*	0.056	2.65*
Error	117	0.005		0.021		0.021	

NOTE: significance levels are given as probability: ^{ns}, P>0.05 and *, P<0.05.
[a]S, side; R(S), rep(side); SL, seed lot; D, duration.

Appendix 8. Effect of the duration of tumbling on percent germination (G), broken seed coats (B), germination + broken seed coats (G+B), and germination + broken seed coats as a function of viability ($G+B^a$) by seed lot.

		G	B	G+B	$G+B^a$
Seed lot	ERC-1	4.8a	7.8abc	12.5a	17.1a
	ERC-2	0.6b	8.8ab	9.3ab	11.6ab
	ERC-3	0.4b	14.5a	15.0a	19.0a
	RMJ-1	0.0b	0.5cd	0.5c	0.6c
	RMJ-2	1.2b	1.8bcd	3.3bc	4.7bc
	RMJ-3	0.2b	1.5bcd	1.8bc	2.7bc
	RMJ-4	0.0b	0.3d	0.3c	0.3c
	RMJ-5	0.0b	0.3d	0.3c	0.4c
Duration (hours)	0	0.8a	2.4a	3.1a	4.0a
	1	1.4a	4.3a	5.6a	7.6a
	2	0.3a	3.9a	4.1a	5.3a
	3	1.5a	5.1a	6.6a	8.8a
	6	0.6a	4.4a	5.0a	6.4a

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$) with respect to transformed data.

Appendix 9. Analysis of variance for germination (G), broken seed coats (B), and germination + broken seed coats (G+B) for the seeds treated with the size-density separation test.

Source of		G		B		G+B	
variation[a]	df	MS	F	MS	F	MS	F
S	1	0.179	16.46*	0.004	0.18^{ns}	0.128	4.73*
SL	4	0.255	23.34*	0.208	9.08*	0.475	17.51*
Z	2	0.017	1.54^{ns}	0.201	8.76*	0.208	7.67*
D	2	0.01	0.87^{ns}	0.146	6.35*	0.149	5.47*
Z x D	4	0.026	2.36^{ns}	0.021	0.92^{ns}	0.033	1.23^{ns}
Error	31	0.011		0.023		0.027	

NOTE: significance levels are given as probability: ^{ns}, P>0.05 and *, P<0.05.
[a]S, side; SL, seed lot; Z, size; D, density.

Appendix 10. Analysis of variance for viability of seeds separated with the Incubation, Dehydration, Separation (IDS) technique. The treatment variable is comparing the viability of the sinkers and the overall viability.

Source of variation[a]	df	MS	F	P > F
SL	4	0.1055	9.96*	0.0001
R	3	0.0081	0.76^{ns}	0.5256
T	1	0.0331	3.12^{ns}	0.0885
SL x T	4	0.0004	0.04^{ns}	0.9963
Error	27	0.0106		

NOTE: significance levels are given as probability: ^{ns}, P>0.05 and *, P<0.05.
[a]SL, seed lot; R, rep; T, treatment.

Appendix 11. Effects of seed lot and seed separation by the Incubation, Dehydration, Separation (IDS) technique on the viability of seeds.

		Overall	Sinkers	Floaters
Seed lot	ERC-1	77.8a	82.5a	12.5a
	ERC-2	73.3a	80a	35a
	RMJ-1	64ab	70ab	30.1a
	RMJ-2	62.5ab	70ab	8.3a
	RMJ-3	48.8b	52.5b	24.8a

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).

Appendix 12. Effects of soaking seeds in deionized distilled water on the leachate conductivity of the water after five hours and microwaving. LI is the leachate conductivity at five hours divided by the leachate conductivity after microwaving.

		5 hours	microwaved	LI
Seed lot	ERC-1	89.21ab	308.03a	28.87b
	ERC-2	118.85a	341.7a	35.47ab
	RMJ-1	40.01b	111.46b	36.07a
	RMJ-2	35.35b	74.46b	47.4a
	RMJ-3	41.75b	64.66b	64.73a

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).

Appendix 13. Analysis of variance for viability of seed lots after germination treatments.

Source of variation[a]	df	MS	F
SL	7	0.429	32.51*
T	5	0.416	31.52*
S(T)	6	0.03	2.24*
T x SL	23	0.027	2.07*
Error	147	0.013	

NOTE: significance levels are given as *, $P<0.05$.
[a]SL, seed lot, T, treatment; S(T), side(treatment).

Appendix 14. Effect of seed lot and treatment on viability of seeds. Control 1 is the viability of seeds straight from the freezer and control 2 is the viability of the control seeds after germination testing.

		Viability			Viability
Seed lot	ERC-1	52.2b	Treatment	Control 1	75.0a
	ERC-2	50.9b		Control 2	53.1b
	ERC-3	72.2a		GA_3	37.8c
	RMJ-1	33.4c		KNO_3	38.5c
	RMJ-2	39.6c		PEG	27.0d
	RMJ-3	39.4c		Separation	30.3cd
	RMJ-4	36.8c		Tumbling	56.6b
	RMJ-5	67.8a			

Means within a column followed by the same letters are not significantly different according to Tukey's studentized range test ($\alpha = 0.05$).